“A study on serum pepsinogen I and pepsinogen II levels and the pepsinogen I/II ratio in Indian patients with gastric cancer”

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DISSERTATION SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT OF THE TAMILNADU DR. M.G.R. MEDICAL UNIVERSITY FOR THE DEGREE OF M.S. BRANCH I - GENERAL SURGERY EXAMINATION TO BE HELD IN MARCH-2009

Certificate

This is to certify that “A study on serum pepsinogen I and pepsinogen II levels and the pepsinogen I/II ratio in Indian patients with gastric cancer”, which is being submitted as thesis requirement for M.S. Degree Branch I – General Surgery examination of the Dr. M.G.R. Medical University of Tamil Nadu, is a bonafide work of the candidate – Dr. Indrani Sen.

Dr. V. Sitaram,

Professor and Head,

Department of General Surgery,

Christian Medical College,

Vellore, Tamil Nadu

Certificate

This is to certify that the topic entitled “A study on serum pepsinogen I and pepsinogen II levels and the pepsinogen I/II ratio in patients with gastric cancer” is a bonafide work done by Dr. Indrani Sen, post graduate in General Surgery of Christian Medical College, Vellore. This work has been carried under my guidance and supervision in partial fulfillment of the regulation of Dr. M.G.R. Medical University of Tamil Nadu for Master of Surgery- Branch I (General Surgery) examination to be held in March 2009.

Dr. George Mathew,

Professor and Head,

Department of General Surgery III,

Christian Medical College,

Vellore, Tamil Nadu

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Contents

Abstract

Background:

The aim of this study was to measure the serum pepsinogen I and II levels in gastric cancer patients at a tertiary care centre in South India and to study the serum pepsinogen I/II ratio in patients with gastric cancer.

Methods:

Thirty three patients: 17 with gastric cancer and 16 normal controls were studied. Serum levels of pepsinogen I (PG I) and pepsinogen II (PG II) were measured by ELISA.

Results:

The mean PG I levels for cancer patients and controls were 185.3 µg/l and 188.1 µg/l, respectively (P= not significant), the mean PG II levels were 21.2 µg/l and 12.7µg/l respectively (P=

not significant). The PG I/II ratio however was 12 in cancer and 25 in controls: the ratio was significantly lower in cancer patients ($P < 0.05$).

Conclusion:

The serum pepsinogen I/II ratio is modified significantly in gastric cancer.

Introduction

Gastric cancer is a leading cause of cancer death in India. India was thought to have lower cancer rates than the Orient. However with the establishment of the National Cancer Registry, the actual disease load is being revealed. Reports from the North Eastern parts of our country show very high rates of carcinoma stomach. Results of treatment are often disappointing because most patients present late. Consumption of large amounts of red chillies, food at very high temperatures, smoked food containing nitrites, preserved food and alcohol consumption are the main risk factors for stomach cancer in India. Consumption of a tobacco extract 'Tuibur' has been linked to the high rates of Stomach cancer in Mizoram. More than 75% of cancers in India present in advanced stages. The 5-year relative survival rates for stage I and stage II stomach cancers are about 77 percent and 48 percent respectively. Stage IV tumours have very low survival rates

around 4% for resectable and even lesser for unresectable tumours. Primary prevention or early detection is the best strategy for prevention and cure of stomach cancer.

Early diagnosis can improve the outcome of gastric cancer, as this disease is curable in the early stages. Screening for early detection of gastric precancerous changes may be helpful in diagnosis and treatment of cancer at curable stages.

Various biomarkers of gastric cancer are under study. Pepsinogen I/Pepsinogen II as markers of atrophic gastritis in corpus, Gastrin-17 as marker of atrophic gastritis in antrum and anti H.Pylori antibodies are all under study in various populations. A large body of work is available from Japan and China.

In 1975, Correa proposed the theory of a consecutive line of events leading to gastric cancer development: normal mucosa - chronic active gastritis - chronic atrophic gastritis - intestinal metaplasia - dysplasia - carcinoma in situ. At present, this

cascade of changes associates with a trigger role of H pylori and is called "Correa's gastric precancerous cascade. A lot of recent research focuses on the role of H.Pylori infection and its role in the pathogenesis of cancer. Chronic Helicobacter pylori (H pylori) infection is thought to be the trigger mechanism in 60-90% of gastric cancer especially in the Orient. Atrophic gastritis also alters the levels of the other enzymes of the stomach: pepsinogen and gastrin. Pepsinogen I and the pepsinogen I/II ratio is being recommended as a screening test for gastric cancer especially in populations at high risk e.g. Japan and China.

This study in our patients is to determine the serum persinogen I/II levels and to examine if there was any alteration in this ratio between patients with normal endoscopy and those with cancer.

Pepsinogen: overview and clinical implications

Pepsinogen is a molecule which has been the focus of recent research in patients with gastric cancer. Human pepsinogens are proenzymes for the digestive enzyme pepsin originating in the gastric mucosa and are classified biochemically and immunochemically into two groups, pepsinogen I and pepsinogen II. These among other functions also act as growth factors and are protective for the gastric mucosa.

One group (Pg1-Pg5), called PGA or group I pepsinogen is characterized by electrophoretically faster migration and is found only in the fundus and body of the stomach. Group I pepsinogens are synthesized solely in the oxyntic glands and mucous neck cells of the gastric corpus. A protein precursor to pepsin, Pepsinogen I or Pepsinogen A is secreted exclusively by the chief cells of the gastric corpus. Serum pepsinogen levels reflect the morphologic and functional status of the

stomach mucosa. Lifestyle, genetic and infectious factors influence the serum levels of this enzyme. This is also influenced by other malignancies and nonmalignant conditions. The levels of Pepsinogen I correlate directly with the active number of chief cells in this area. In cases of severe atrophic gastritis of the corpus region, the chief cells will be destroyed and the levels of Pepsinogen I will fall. The majority of pepsinogens are secreted into the lumen of the stomach where they are metabolized into active pepsin. Small proportions about 1% of the pepsinogens diffuse into the blood circulation.

The second group (Pg6 and Pg7), termed pepsinogen C or group II pepsinogen, is localized in the whole stomach. It is also produced at sites distant from the gut, specifically, the pancreas, prostate, seminal vesicle, and lung. Pepsinogen C was also found to be a marker of breast cancers, with a more favorable response to hormone-based chemotherapy. This led to studies of the hormone responsiveness of a 1,438-base

segment of the pepsinogen C promoter, which demonstrated a 15-bp hormoneresponsive element that resembles the consensus sequence for glucocorticoid, androgen, and progesterone receptors. Synthesis of PGC has also been reported in numerous non-gastric tissues including the prostate gland and the Brunner's gland of the small intestine. The physiological significance of PGC in these tissues is not known and studies are limited by the lack of appropriate in vitro cell models. Animal models are under development. Serum concentration of PG II reflects the histological status of gastric mucosa.

The ratio of concentration of PGI to PGII in serum of normal subjects is about 4:1. In case of atrophic corpus gastritis, the level of serum pepsinogen I decrease whereas the level of pepsinogen II remains stable or decreases slightly. The PGI/PGII ratio decreases linearly with increasing grade of atrophic gastritis in the corpus (2, 3). The ratio is < 2.5 when the

atrophic gastritis is advanced (moderate or severe) in the gastric corpus. A combination of the serum pepsinogen I level and the pepsinogen I/II ratio has recently been recommended to screen for gastric cancer

Very few studies have been done in India. Serum pepsinogen (SP) levels were studied in 100 patients with gastroduodenal lesions, and 100 healthy volunteers. SP levels were significantly elevated in patients with duodenal ulcer (DU) and duodenitis compared to the controls. SP values above 150 ug Tyr/ml/24 hr were highly suggestive of duodenal ulcer disease. The mean values of SP in North India were found to be lower in both normal and DU subjects compared to the west.

Another study concluded that serum pepsinogen estimation is a useful diagnostic and screening tool in the diagnosis of carcinoma stomach. This study showed that patients with carcinoma stomach had a significantly lower pepsinogen I level

(87.2ug/L) and pepsinogen I/II ratio (4.3). The cut-off levels of pepsinogen I (115.3ug/L) and pepsinogen I/II ratio (6.2) when applied in parallel provided a sensitivity of 97% and a negative predictive value of 91.4%.

Various cutoff values for screening have been proposed; typically, serum pepsinogen I less than 70µg/l and a PG I/II ratio of less than 3 are used for screening in other populations. The sensitivity and specificity ranged from 55% to 84.6% and 73.5% to 78.9%.

The difference in the results in these populations can have various reasons. First, there is a difference in prevalence of gastric atrophy: this may be low in our population. Also, gastric atrophy is not always an essential intermediate step in gastric carcinogenesis. Different populations develop cancer due to a combination of factors.

In the analysis by location, cancer found in both the proximal one third and the distal two thirds of the stomach showed a significant association with serum pepsinogen test levels; however, the magnitude of the association was stronger for body/distal cancer. Atrophic change in gastric mucosa spreads from the anal end to the oral end. Thus, the association between serum pepsinogen test levels and development of gastric cancer is considered stronger for distal cancer. This finding was in accord with those from previous case-control studies.

With regard to depth of invasion, both early and advanced cancers were clearly associated with serum pepsinogen test levels. Some studies however did not detect early cancers.

Men have a higher risk of developing gastric cancer after severe gastric atrophy and serum pepsinogen levels mimic this. In animal experiments, this tolerance of women toward

carcinogenesis has been explained in terms of their sex hormones. When the serum pepsinogen test is used to screen for gastric cancer, it might be preferable to vary the cutoff level according to sex.

The lower incidence of gastric cancer among Indians as compared to Chinese cannot be explained by differences in H. pylori or serum PG. Other modifying factors such as genetic factors may be important.

Genetics of pepsinogen

The absence of detectable immunologic crossreactivity between the pepsinogens A and C results from divergent evolution of sequences located on the surface of the zymogens in contrast to the strongly conserved active site regions located within the binding cleft of the enzymes, inaccessible for antigenic recognition. Although antigenically distinct, pepsinogens A and C exhibit a high degree of homology in their active site regions. Hayano et.al. isolated the entire human pepsinogen C gene from a cosmid genomic library. Pepsinogen genes comprise nine exons and may be multiple, especially for pepsinogen A. The latter and progastricsin predominate in adult animals, while pepsinogen F and prochymosin are the main forms in the fetus/infant.

The switching of gene expression from fetal/infant to adult-type pepsinogens during postnatal development is noteworthy, being regulated by several factors, including steroid hormones.

Pepsinogen C is a type 2 cell-specific marker that exhibits tight developmental regulation in vivo during human lung development, as well as during in vitro differentiation and dedifferentiation of type 2 cells.

PGA is the precursor of pepsin A. PGC, also known as progastricsin, is the precursor of pepsin C or gastricsin. PGA has five electrophoretic isozymogens (Pg 1-5). PGA is remarkably heterogeneous as shown by an extensive protein electrophoretic polymorphism resulting in multiple haplotypes containing different combinations of the individual PGA genes and one or more post-translational modifications of the primary gene products. Two principal forms of PGA polymorphism have been recognised: phenotype A, which possesses all five PGA isozymogens; and phenotype B, which lacks Pg 5.

The PGC gene was localized to human chromosome 6p21.1-pter by analysis of mouse x human somatic cell hybrids. PGC

has two electrophoretic isozymogens (Pg 6 and Pg 7). No genetic variation has been described at the protein level. However at the DNA level, a 100 bp insertion-deletion RFLP located between exons 7 and 8 was observed with several restriction enzymes. The RFLP for PGC was identified in Indian, Caucasians and Japanese. Whether this polymorphism could affect the expression of PGC gene or regulate the PGC gene expression when the stomach was attacked by some pathogenetic factors is not known. Another possible explanation of the association between the PGC gene polymorphism and gastric cancer was that the PGC gene was not itself responsible for the predisposition but one of the responsible genes was closely linked to it.

PCG gene polymorphisms have also been shown to be associated with gastric body cancers and this is another exciting new field of research. There is a significant association between genetic polymorphisms at the PGC gene locus and

gastric ulcer disease and that there is genetic heterogeneity in this disease; this depends on the location of the gastric ulcer. The PGA: PGC ratio was significantly lower in patients with gastric body ulcer than in patients with duodenal ulcer and in patients with angular or antral ulcer. Ulcers may be more proximal when atrophic gastritis is more severe. These findings suggest that the different pathophysiological factors between gastric body ulcer and gastric angular or antral ulcer may have underlying genetic differences.

This is a field open for future research and identification of gene mutations predisposing to cancer may be used as diagnostic tools.

Biochemistry of pepsinogens

Five types of zymogens of pepsins, gastric digestive proteinases, are known: pepsinogens A, B, and F, progastricsin, and prochymosin. The amino acid and/or nucleotide sequences of more than 50 pepsinogens other than pepsinogen B have been determined to date. Phylogenetic analyses based on these sequences indicate that progastricsin diverged first followed by prochymosin, and that pepsinogens A and F are most closely related. Tertiary structures, clarified by X-ray crystallography, are commonly bilobal with a large active-site cleft between the lobes. Two aspartates in the center of the cleft, Asp32 and Asp215, function as catalytic residues, and thus pepsinogens are classified as aspartic proteinases.

Conversion of pepsinogens to pepsins proceeds autocatalytically at acidic pH by two different pathways, a one-step pathway to release the intact activation segment directly,

and a stepwise pathway through a pseudopepsin(s). The active-site cleft is large enough to accommodate at least seven residues of a substrate, thus forming S4 through S3' subsites. Hydrophobic and aromatic amino acids are preferred at the P1 and P1' positions. Interactions at additional subsites are important in some cases, for example with cleavage of 3-casein by chymosin. Two potent naturally occurring inhibitors are known: pepstatin, a pentapeptide from Streptomyces, and a unique proteinous inhibitor from Ascaris.

Gastrin

Another molecule which can be targetted as a diagnostic test is gastrin. Gastrin is synthesized in G-cells, which are found in the gastric antrum. The gastrin secreted by the antrum is over 90% of type G-17 whereas the gastrin secreted by the duodenum is primarily type G-34. The fasting serum gastrin is primarily in the form of G-34 but the proportion of type G-17 increases after the dietary stimulus. The secretion of gastrin-17 can be studied with a simple protein stimulation test. First, a blood sample is taken after fasting, after which the patient eats a protein-rich meal. The maximum increase in the level of gastrin-17 can be seen in the serum within 20 min. If the serum gastrin does not increase as a result of protein or other physiological stimulation it is an indication of the loss of gastrin secreting G cells, i.e., an indication of the atrophy of the antrum mucosa. It is possible to make indirect conclusions of the status of the antrum mucosa by simultaneously assaying the serum

gastrin and acid output. In the cases with atrophic antral gastritis and loss of antral G cells, serum gastrin remains low although the stomach is achlorhydric or hypochlorhydric.

H. pylori eradication may cure gastritis and help to prevent further progression of gland loss and reduce the risk for gastric cancer in populations where its correlation with cancer is high. Screening and treatment of H. pylori infection might in theory be cost-effective for the prevention of gastric cancer in such populations.

Patients and methods

This study was a case control study done on a population of

hospital based patients at a tertiary care centre in South India. Specimens and data were collected prospectively from December 2006 to December 2007.

Forty three patients were diagnosed to have carcinoma stomach in the period December 2006 to December 2007. Seventeen of them were chosen randomly as study cases. The control group comprised of an equal number of patients. Control subjects were selected from among those who had no endoscopic abnormalities on evaluation for dyspepsia. Patients were interviewed with a questionnaire; data recorded in proformas and blood samples were collected for assay.

Inclusion criteria were patients with malignant gasric ulcers on endoscopy.
Exclusion criteria were patients with previous gastric surgery or any other treatment for stomach cancer, such as chemotherapy or radiotherapy.

Informed consent was obtained from all patients before the data was collected.

Serum pepsinogens were measured by using pepsinogen I and II Biohit kit. This is manufactured by the Biohit Diagnostics and is commercially available.

Methods

Sample collection

Patients were fasting for 10 hours before blood sampling. Blood sample was collected by venipuncture into a plastic EDTA tube without additives. Plasma blood tubes were mixed immediately by turning them upside down 5-6 times and tubes for serum allowed to clot (for minimum 30 minutes) at room temperature (20...25°C). Serum after clotting was separated by centrifugation and stored frozen_20°C. Samples were mixed thoroughly after thawing. Grossly hemolysed, lipemic or turbid specimens were avoided and samples were not repeatedly froze and thawed.

Working principle of the ELISA

The pepsinogen kit is a microplate-based quantitative enzyme linked immunosorbent assay (ELISA) for the determination of human pepsinogen from plasma or serum samples. This ELISA is based on a sandwich enzyme immunoassay technique with a PG specific capture antibody adsorbed on a microplate and a detection antibody labeled with horseradish peroxidase(HRP).

The assay proceeds according to the following reactions:

1. A monoclonal antibody, specific to human PG, on the polystyrene surface of the wells binds PG molecules present in the sample.

2. Wells are washed to remove the residual sample.

3. An HRP-conjugated monoclonal detection antibody is added to the wells and it binds to the PGI molecules.

4. The wells are washed and TMB-substrate is added. The substrate is oxygenized by the enzyme and a blue colored end product is produced.

5. The enzyme reaction is terminated with stop solution. The solution in the microwells should turn yellow. The intensity of the yellowish color developed is directly related to the PG concentration of the sample.

Details of the Kit:

The reagents are sufficient for 96 wells and three separate runs.

1. Microplate

Contents: 12 x 8 strips in frame coated with high-affinity, monoclonal anti-human -PGI IgG1.

2. Washing Buffer Concentrate (x 10)

Contents: 120 ml of 10 x phosphate buffer saline (PBS) concentrate containing Tween 20 and 0.04% ProClin 300 as preservative.

Preparation: Dilute 1 to 10 (e.g. 100 ml+ 900 ml) with distilled water and mix well.

Stability: The diluted solution is stable for two weeks refrigerated (2…8 °C).

3. Diluent Buffer

Contents: 100 ml of phosphate buffer containing bovine serum albumin, Tween 20, 0.04% ProClin 300 as preservative and red dye extract.

4. Blank Solution

Contents: One vial containing 1.5 ml of human serum-based phosphate buffer with 0.1% ProClin 300 as preservative.

5. Calibrators

Contents: Three vials each containing 1.5 ml of human serum-based calibrators with 0.1% ProClin 300 as preservative.

6. Control

Contents: One vial containing 1.5 ml of human serum-based PGI control with 0.1% ProClin 300 as preservative.

6.7. Conjugate Solution

Contents: 15 ml of HRP-conjugated monoclonal anti-human-PGI in stabilizing buffer with 0.02% methylisothiazolone, 0.02% bromonitrodioxne and 0.002% other active isothiazolones as preservatives.

8. Substrate Solution

Contents: 15 ml of tetramethylbenzidine (TMB) in aqueous solution.

9. Stop Solution

Contents: 15 ml of 0.1 mol/l sulphuric acid.

10. Incubation Covers

11.Other materials

1. Distilled or deionized water
2. Micropipettes and disposable tips
3. Pipettes to accurately deliver 1 - 10 ml
4. 8-channel pipette delivering 100 µl
5. Graduated cylinder, 1000 ml
6. Vortex mixer for sample dilutions
7. Test tubes for specimen dilutions
8. Microplate washer
9. Paper towels or absorbent paper
10. Incubator, 37°C
11. Microplate reader, 450 nm

Storage and transport

The Pepsinogen kit was stored and transported refrigerated (2…8°C) till use. Liquid components were clear. The substrate

solution was pale red. (Any other color indicates deterioration of the substrate solution.)

Test procedure

Preliminary preparations

Allow all reagents and the microplate to reach room temperature (20…25°C). Warm the incubator to 37°C. Dilute the washing buffer concentrate 1 to 10 (e.g. 100 ml + 900 ml) with distilled or deionized water.

Specimen dilution

Dilute serum or plasma samples 1 to 10 (50 µl + 450 µl) with the diluent buffer, mix well.

STEP I

Mix and pipette 100 µl of the blank solution (BS), the calibrators (CAL1-CAL3), the control and diluted samples (S1, S2 etc.) into the wells as duplicates (see Figure 1). Cover the plate with the incubation cover. Incubate for 60 minutes at 37°C.

WASHING

Wash the wells three times with 350 µl of the diluted (1 to 10) washing buffer and gently tap the inverted plate a few times on a clean paper towel.

STEP II

Pipette 100 µl of the mixed conjugate solution into the wells, preferably with an 8-channel pipette. Cover the plate with the incubation cover. Incubate for 30 minutes at 37°C.

WASHING

Wash the wells three times with 350 µl of the diluted (1 to 10) washing buffer and gently tap the inverted plate a few times on a clean paper towel.

STEP III

Pipette 100 µl of the mixed substrate solution into wells with an 8-channel pipette. Start the incubation time after pipetting the substrate solution into the first strip and continue the incubation for 30 minutes at room temperature (20…25°C). Avoid direct exposure to light during incubation.

STEP IV

Pipette 100 µl of the mixed stop solution with an 8-channel pipette into the wells.

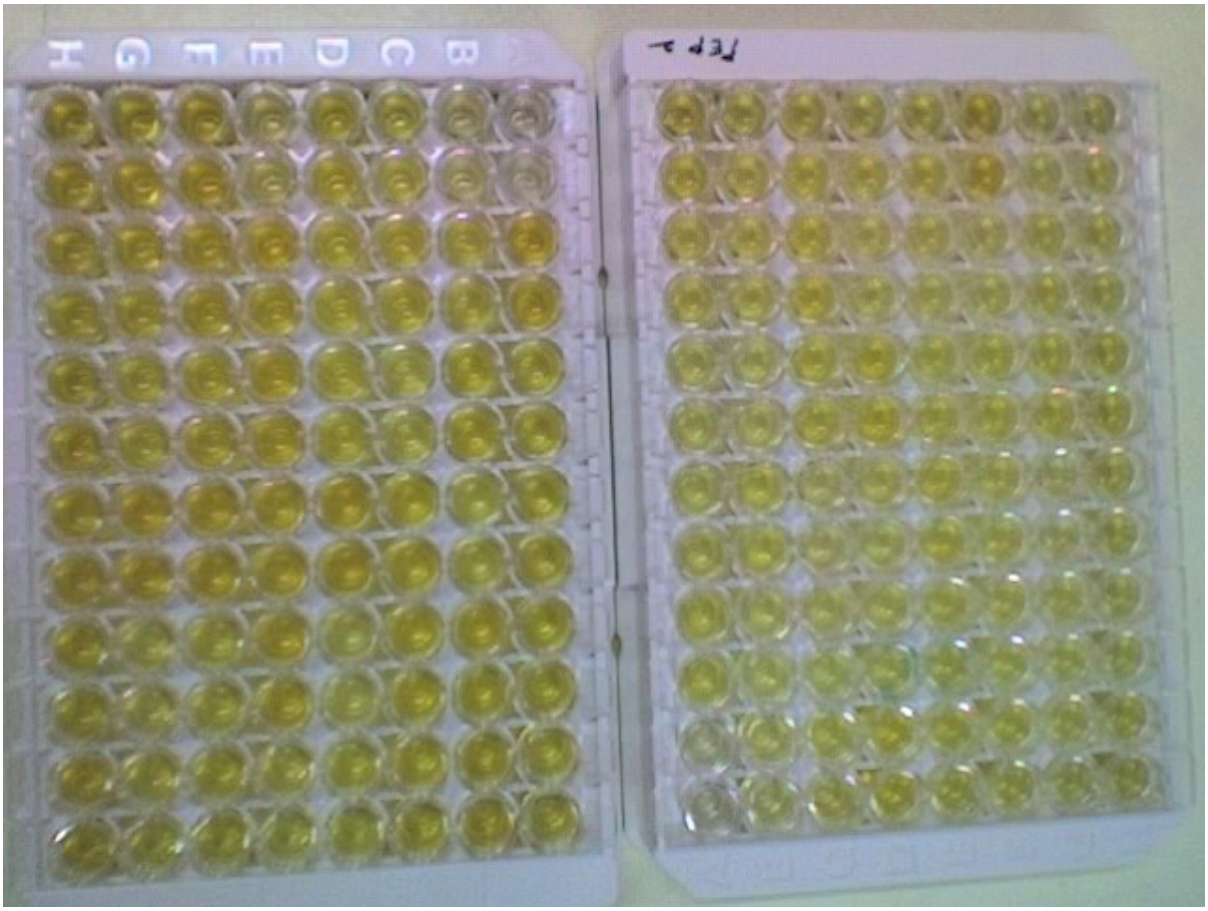
MEASURING

Measure the absorbance at 450 nm within 30 minutes.

A low P/S-PGI result (P/S-PGI<25 µg/l) indicates advanced atrophic gastritis of the corpus mucosa. This cut-off level has been determined using the Biohit Pepsinogen I ELISA kit based on large clinical material.

The test kit

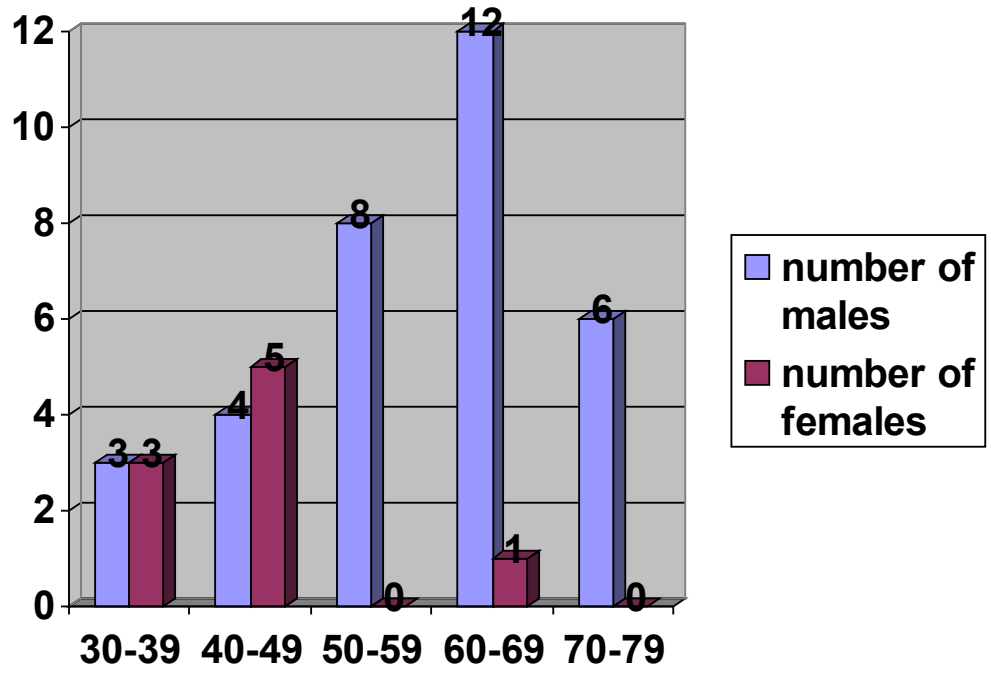
Microplates at analysis



Results and analysis

Forty three patients were diagnosed to have carcinoma stomach in the period December 2006 to December 2007. There were 34 male and 9 female patients.

Table 1: Age and sex distribution of patients from Dec 06-07



Seventeen of them were chosen randomly as cases: there were no similar Indian studies at the time the study commenced: sample size was indirectly calculated based on the available Indian studies.

Seventeen patients with gastric cancer (mean age 58.3 years; range, 31-74 years) and 17 age matched control subjects were studied. There were 15 male and 2 female cancer patients- the male:female ratio was 7.5:1.

Table 2: Male: female distribution of study patients

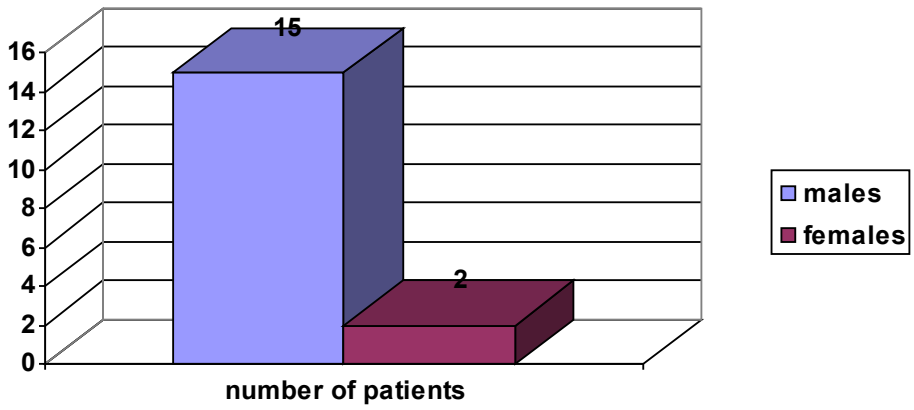
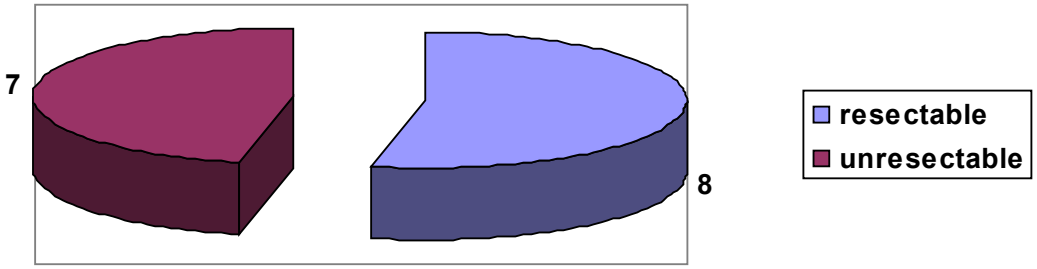


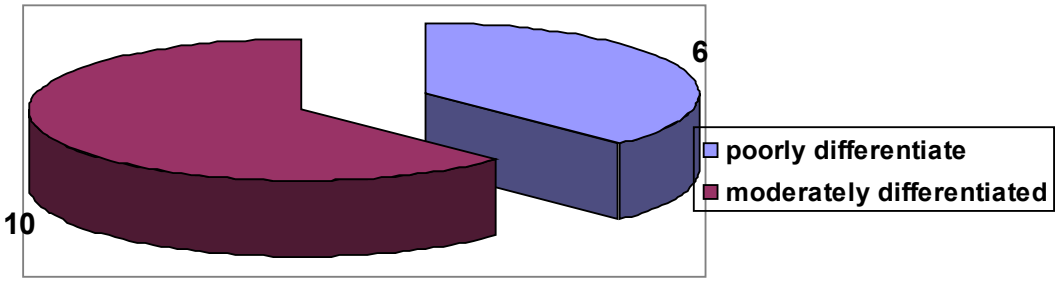
Table 3: Resectability at diagnosis in study patients



Seven out of 15 tumours were unresectable at diagnosis. There were 6 poorly differentiated and 10 moderately differentiated tumours. Tissue sample was not adequate for grading in one patient.

Of the unresectable tumours, only 2 were poorly differentiated: majority was moderately differentiated (5/7).

Table 4: Tumour histology in study patients



Three patients (2male, 1 female) with operable tumours did not undergo any further treatment at this centre after diagnosis.

Seven patients underwent resection. Four underwent subtotal gastrectomy and 3 underwent distal gastrectomy.

Four patients were followed up and of these three were well at 7 months. Two had undergone subtotal and one distal resection with tumour free margins on histology: they had moderately differentiated adenocarcinoma. One patient had

evidence of nodal recurrence within a month and he had a poorly differentiated tumour.

Intestinal metaplasia and gastric atrophy was not reported in any of the study patients.

Serum levels of pepsinogen I, pepsinogen II, and the PG I/II ratio in the cancer patients and controls is depicted in the following tables.

Table 5: Levels of Pepsinogen I, II and the I/II ratio in cancer patients

Patients	Pepsinogen I ug/l	Pepsinogen II ug/l	Pepsinogen I/II ratio
1.	232.4	23	10.11
2.	216.9	12.2	17.7
3.	188.7	22.9	8.2
4.	234.6	14	16.7
5.	224.6	24.6	9.1
6.	146.1	17.6	8.3
7.	100	22.8	4.3
8.	196.3	63.4	3.1
9.	66.6	20.2	3.2
10.	225.6	18.2	12.3
11.	121.1	5.7	21.2
12.	233.8	9.3	25.1
13.	59.7	5.9	10.1
14.	228.8	32.5	7.04
15.	227.4	8.7	26.1
16.	212	11.8	17.9
17.	234.9	47.7	4.9

Table 6: Levels of Pepsinogen I, II and the I/II ratio in controls

Controls	Pepsinogen I	Pepsinogen II	Pepsinogen I/II ratio
1.	210.7	12.5	16.8
2.	171.1	9.6	17.8
3.	215.3	17.3	12.4
4.	80.7	2.6	31
5.	167.1	6.2	26.9
6.	231.3	30.5	7.5
7.	203.8	13.7	14.8
8.	139.9	2.3	60.8
9.	191.9	9	21.3
10.	227.7	9.8	23.2
11.	229	15.1	15.16
12.	161.6	7.9	20.4
13.	182.7	4.9	37.2
14.	134.6	4.8	28
15.	235.1	22.3	10.5
16.	226.4	35.4	63.9
17.	234	17.8	13.1

Statistical analysis

These results showed serum pepsinogen I values that were very high in comparison to the values in other populations.

The statistical test used was the significance of the normal deviate and the standard error of deviation of two means. There was no satistically significant difference in the levels of PepsinogenI or II when tested individually. However, the Pepsinogen I/II ratio was significantly different between the two groups.

The normal serum I/II ratio is thought to be around 4:1 and this falls to below 2.5:1 in patients with gastric atrophy and cancer. The average levels of Pepsinogen I is between 50-150ug/l and Pepsinogen II around 15 –20ug/l. In our paients the

corresponding levels were 185ug/l and 21ug/l respectively: these values were much higher than what has been reported in literature. The average pepsinogen I/II ratio was 12 in patients and 25 in controls which is much higher than what is reported in other populations..

Using a cutoff value of 7 for the pepsinogen I/II ratio and applying the test yields a sensitivity of around 47% but specificity of 100%. The positive predictive value of the test at this cutoff is 100%. Other Indian reports have also recommended similar cutoff values.

The other clinical or pathological variables had no effect on serum pepsinogen levels.

Discussion

Gastric cancer is the second most common cancer in the world. The mortality of gastric cancer is still in the leading status of all cancers. The 5-year survival rate of gastric cancer is low, and identification and a better control of risk factors seem to be the most effective means of prevention.

Screening for early detection of gastric precancerous changes may be helpful in diagnosis and treatment of cancer at curable stages.

Many factors ascribe to the cause of gastric cancer, including the living habit, nutrition, microbe, and genetic predisposition. Recently, following the primary completion of Human Genome Project, the association of genetic polymorphisms with diseases came to the study frontier. Genetic polymorphisms are defined as variations in DNA that are observed in 1 % or more of the population. The study of genetic polymorphisms promises to

help define pathophysiologic mechanisms, to identify individuals at risk for disease and to suggest novel targets for drug design and treatment. Family members of gastric cancer patients have been found to have a 1.5-fold to 3-fold increase in the risk of developing this cancer. This familial aggregation may be due to genetic or environmental factors shared by family members.

H.pylori is considered a WHO class I carcinogen. Chronic H. pylori gastritis, in more than half of the affected subjects, could lead to a gradual loss of glandular structures with its specialized cells and a collapse of the reticulin skeleton of the mucosa, a condition of atrophic gastritis. As a result, the glandular layer of the mucosa became thinner, and glands were replaced by fibrosis and intestinal metaplasia. The major clinical importance of this condition was that it could significantly increase the risk for the intestinal type of gastric cancer. This

risk might be elevated up to 90- fold in subjects with severe atrophic gastritis throughout the complete stomach. The annual incidence of gastric cancer among patients with atrophic gastritis varied in cohort studies between 0.3 and 1.0%. This could explain the interest in the diagnosis of atrophic gastritis. At present, there is a wide circle of questions related to the diagnosis of critical stages of gastric carcinogenesis - gastric epithelial atrophy, intestinal metaplasia and dysplasia. Therefore, it is extremely important to recognize dyspeptic patients who have very high risk of gastric malignant changes and require dynamic surveillance with the purpose of early revealing of the preneoplastic changes in stomach mucosa. Atrophic gastritis is a serious disease, which often does not receive much attention. The relationship between gastritis, atrophic gastritis and other diseases of the stomach is based on the fact that infection and atrophy could alter the physiological functions of the stomach and influence the growth and growth control of epithelial cells in the stomach. These consequences

varied depending on whether the changes of the gastric mucosa caused by gastritis were located in the antrum or the corpus or both.

H. pylori infection is known to be associated with a raised serum pepsinogen II level and a decreased PG I/II ratio. Expression of cytotoxin-associated gene A (cagA) which is known to be associated with more active mucosal inflammation and, hence, higher levels of serum pepsinogen I and II than other strains. However the incidence of cancer is the same in cagA positive and negative patients.

However not all cancers are associated with atrophic gastritis. The most accurate diagnostic method of gastrointestinal tract diseases is endoscopy with subsequent biopsy, which should be made in all patients with the presence of clinical symptoms. However, because of patchy characteristics of atrophic changes in stomach mucosa, some histological researches could give false - negative results. Due to invasiveness of

biopsy, it is expedient to make only for monitoring precancerous changes in stomach mucosa.

For the selection of patients recommended to biopsy, the presence of a screening method is necessary. Ohata et al. assessed H. pylori–negative subjects and found an increased risk of gastric cancer for those with a positive pepsinogen test level (pepsinogen I ≤70 ng/ml and pepsinogen I/II ratio ≤3.0) compared with subjects with a negative level. It is reasonable that pepsinogen test positivity, a marker of chronic atrophic gastritis, is closely associated with this type of gastric cancer. On the other hand, diffuse-type gastric cancer is thought to be genetically determined, at least in part, and to be less associated with environmental factors. This type of cancer does not progress through severe atrophic gastritis; hence, no clear association with the serum pepsinogen test is seen.

In our study, the baseline levels of Pepsinogen as well as the cutoff for PG I/II ratio was significantly higher. This might be explained by a low incidence of atrophic gastritis in our population. Other etiopathologies assume greater significance in this setting.

Noninvasive detection of gastric mucosal atrophy by means of enzyme immunoassay with assessment of G-17 and PG1 levels can be offered as the screening tool for gastric precancerous conditions. On the other hand, this method does not diagnose intestinal metaplasia and cancer development in stomach mucosa. Therefore, the results of serological screening indicating the stomach mucosal atrophy require carrying out the chromoendoscopy with subsequent mucosal biopsy, for revealing probable progressing of atrophic process with development of intestinal metaplasia, dysplasia or gastric cancer.

Cost of screening and the ethical implications of screening also have to be considered carefully in a developing country like India.

This study is limited by the number of patients studied: larger studies are needed before this test becomes clinically applicable.

Conclusion

Favourable patient outcome in the management of carcinoma stomach still depends on early detection. At present endoscopy is the gold standard of diagnosis. Screening tools are under development but there is no current test which can replace endoscopy. These also have to take into account the different nature of the disease in Indian patients as well as cost.
Our study evaluates a test which has been recommended as a alternative or adjunct to the diagnosis of gastric cancer. We find a significant difference in test results in cancer patients.

In future further subset analysis by location of cancer, enzyme levels between different communities, temporal change of enzyme levels with progression of disease also needs to be studied. Studies with larger numbers of patients are needed for validation of this test before routine clinical use.

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Appendix

Study proforma for cases

Form no.

Name **Hospital number :**

Age : **Address**

Sex : **Telephone number:**

Date of detection:

Risk factors (circle if present)

1. Smoking
2. Alcohol
3. Documented H. pylori
4. Previous gastric surgery (if yes, details and date)

5. Pernicious anemia
6. Menetriers disease
7. Blood group A
8. Family history
9. Diet - Smoked fish / spices / tea

Socio economic status: : Poor / not poor

Known past history of ulcer disease: Yes ___years / No

Comorbidity

Details of family history

Family tree

Symptoms

Symptoms	yes	no
Weight loss		
abdominal pain		
Anorexia		
Vomiting		
Dysphagia		
Haematemesis		
Malena		
Non specific dyspepsia		

Clinical examination

Finding	yes	no
Palpable abdominal mass		
Jaundice		
Ascites		
SCLN		
Rectal deposits		

Pre op investigations:

Blood **Hb :**

Endoscopy:

	Distance in Cm
GE junction at	
Growth extending from	
Growth extending to	
Pylorus	
Duodenum	

Radiological investigations:

Biopsy (endoscopic)

Surgery done :

Operative findings :

Primary tumour

Regional nodes

Distant metastasis

Bormann type : 1/ 2 / 3 / 4 **Laurens type :**

Intestinal / Diffuse

Post op biopsy :

Histology : Well / moderately / poorly differentiated adenocarcinoma

Sub type : Papillary / tubular / mucinous / signet cell /others

Margins : Proximal -

Distal -

Nodes :

Size of tumor :

TNM stage :

AJCC stage :

Study proforma for controls

Form no.

Name **Hospital number :**

Age : **Address**

Sex : **Telephone number:**

Date of detection:

Risk factors (circle if present)

1. Smoking
2. Alcohol
3. Documented H. pylori
4. Previous gastric surgery (if yes, details and date)
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7. Blood group A
8. Family history
9. Diet - Smoked fish / spices / tea

Socio economic status: : Poor / not poor

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Comorbidity

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Family tree

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abdominal pain		
Anorexia		
Vomiting		
Dysphagia		
Haematemesis		
Malena		
Non specific dyspepsia		

Clinical examination

Finding	yes	no
Palpable abdominal mass		
Jaundice		
Ascites		
SCLN		
Rectal deposits		

Pre op investigations:

Blood **Hb :**

Endoscopy:

	Distance in Cm
GE junction at	
Growth extending	

from	
Growth extending to	
Pylorus	
Duodenum	

Radiological investigations:

Biopsy (endoscopic)

Informed consent form

Title of the study : Levels of serum pepsinogen I and II in patients with carcinoma stomach and the genetic polymorphisms of pepsinogen C associated with carcinoma stomach in the Indian population.

Institution **:** Christian Medical College

Nature and purpose of the study: you are taking part in studies that will asses the level of a particular enzyme in your

blood. This enzyme is altered in many diseases affecting the stomach. Indian patients may have different enzyme patterns. However this has so far not been studied in India. Your blood enzyme will be studied and compared to other patients to see if there is any significant difference.

You will have to give 8 ml blood sample after undergoing your gastroscopy. There is no appreciable risk of giving this small blood sample. The records from this study will remain strictly confidential at all times. It will be available only to the doctors and researchers conducting the study. You can refuse to be a part of this study and this will not affect your future medical care in any way.

Consent:

I have read /had read out to me the above information and give informed consent after understanding it.

Signature of the patient:

Signature of the witness:

Patient name :

Hospital number :

Consent form no. :